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TECHNICAL ARTICLE

Methods to mark termites with protein for mark–release–recapture and mark–capture type studies

J. R. Hagler · P. B. Baker · R. Marchosky · S. A. Machtley · D. E. Bellamy

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Abstract Studies were conducted to investigate the feasibility of marking the southwestern desert subterranean termite, *Heterotermes aureus* (Snyder), with rabbit immunoglobulin G (IgG) protein for mark–release–recapture (MRR) and mark–capture type studies. Qualitative laboratory studies were conducted to determine how long reagent-grade rabbit IgG is retained on or in *H. aureus* that were marked either externally with a topical spray, internally by feeding them a rabbit IgG-marked food source, or both internally and externally (double marked). Marked termites were detected by an anti-rabbit IgG enzyme-linked immunosorbent assay. Data indicated that the termites retained the mark for at least 35 days, regardless of the marking procedure. A second series of laboratory studies were conducted to determine how fast *H. aureus* acquire the mark after feeding on cardboard bait that was either sprayed or soaked in different formulations of rabbit IgG. The IgGs tested were a highly purified and costly reagent grade IgG at 5.0 mg/ml and a less pure and less costly technical grade rabbit IgG at 1.0 mg/ml. The results showed that termites acquired both marks equally well after exposure to the soaked cardboard treatment. The advantages and limitations of protein marking termites with rabbit IgG for MRR or mark–capture termite studies are discussed.

Keywords *Heterotermes aureus* · Protein marking · ELISA · Trophallaxis · Rabbit IgG

J. R. Hagler (✉) · S. A. Machtley
USDA-ARS, Arid-Land Agricultural Research Center,
21881 N. Cardon Lane, Maricopa, AZ 85238, USA
e-mail: James.Hagler@ars.usda.gov

S. A. Machtley
e-mail: Scott.Machtley@ars.usda.gov

P. B. Baker · R. Marchosky
University of Arizona, Tucson, AZ 85721, USA
e-mail: pbaker@ag.arizona.edu

R. Marchosky
e-mail: rjmarcho@ag.arizona.edu

D. E. Bellamy
ED&A Consulting, Buckley, WA 98321, USA
e-mail: dave.bellamy@gmail.com

Introduction

Understanding the basic biology and ecology of subterranean termites is essential for developing reliable management practices. A critical factor for effectively controlling termites is thorough knowledge of their dispersal patterns. Historically, termite dispersal studies have used mark–release–recapture (MRR) and mark–capture type techniques (Hagler and Jackson, 2001). For MRR studies the termites are first collected in the field or obtained from a laboratory colony, marked, and then released into the field (Esenther, 1980; Forschler and Townsend, 1996). In turn, termites are recaptured at given temporal and spatial intervals after their release to determine if they possess the mark. Studies using the mark–capture technique begin by placing dye-marked cellulose-based feeding stations in the field. Termites obtain the mark internally by feeding on the dye-marked paper and then are captured (Su, 1994). These two common techniques have been used to assess the foraging territories of many termite species (Jones et al., 1987; Jones, 1990; Su et al., 1993; Su, 1994).

The key component to any termite dispersal study is an effective marker. An ideal marker should be easy to apply and identify, durable, cost effective, and non-toxic to the

arthropod and environment (Hagler and Jackson, 2001). For termites, the most successful markers used to date have been oil-based dyes (Grace and Abdallay, 1989; Jones, 1990; Su et al., 1991; Haagsma and Rust, 1993). Various dyes have been shown to have either long or short retention on various termite species (Su et al., 1991; Thorne et al., 1996), which can be an asset or a liability, depending on the objective of the study.

Hagler and Jackson (2001) reviewed the current methods available for marking arthropods. They discussed the potential for marking arthropods with exogenous proteins. Hagler et al. (1992) were the first to show that it is feasible to mark arthropods with a foreign protein such as rabbit immunoglobulin G (IgG) that is then identified by a sensitive protein-specific enzyme-linked immunosorbent assay (ELISA). Since then, IgG marks have been applied to study the dispersal characteristics of a wide variety of insects (DeGrandi-Hoffman and Hagler, 2000; Hagler et al., 2002; Hagler and Naranjo, 2004; Blackmer et al., 2004; Peck and McQuate, 2004; Buczkowski and Bennett, 2006). Recently, Buczkowski et al. (2007) successfully used reagent grade rabbit IgG as a mark to examine trophallaxis and feeding relationships in the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), under laboratory conditions.

The present laboratory studies investigate the feasibility of using rabbit IgG to mark the desert subterranean termite, *Heterotermes aureus* (Snyder), which is among the most important pests of structures in the Southwestern United States. The first study was conducted to determine how long a rabbit IgG mark is retained in or on *H. aureus*. We marked termites either: (1) externally (for use in MRR-type studies) with a topical spray, (2) internally (for use in mark–capture type studies) by feeding them a "reagent grade" rabbit IgG-marked food source (e.g., bait) or (3) both internally and externally (double marked). Cohorts of marked termites were then analyzed by ELISA for ≥35 days after marking to detect the presence of the mark. Additional laboratory studies were then conducted to test for differences in internal marking efficiency by feeding termites cardboard that was either sprayed or soaked in different purities of rabbit IgG. The IgGs tested were a highly purified and costly ($8.21 per mg) reagent grade IgG and a less pure and less costly ($5.60 per mg) technical grade rabbit IgG. The advantages and limitations of this technique for MRR or mark–capture termite studies are discussed.

Materials and methods

Long-term retention of protein on termites

A series of studies were conducted to determine how well *H. aureus* retains an internal, external, or an internal plus external (double) protein mark. The termites originated from collections made at The University of Arizona, Santa Rita Experimental Range, Pima Co., Arizona, USA (elevation 984 m, GPS coordinates N 31.88397: W 110.88375). The main termite colony was maintained in a 1.9 l white plastic bucket that contained a 7-cm diameter × 8-cm tall roll of corrugated cardboard as a food source (0.04 × 1.0-m strip of CR 30 × 250 B-flute SF cardboard, Tucson Container Corp, Tucson, Arizona, USA). The colony was kept in total darkness in an incubator (Percival Scientific Inc, Model I-36LL, Perry, Iowa, USA) set at 21°C and 90% RH. Termites were removed from the main termite colony and then divided into four treatment groups consisting of ≈500 individuals each. Each of three of the termite groups was marked either externally, internally, or internally plus externally (double marked) with rabbit IgG. The final group of termites was reserved as an unmarked control treatment group (see below).

Internal marking procedure

A 150-mm diameter No. 1 Whatman filter paper was sprayed with 3.0 ml of a 5.0 mg/ml reagent grade rabbit IgG solution (Sigma, St. Louis, Missouri, USA, cat. # I-5006) using a standard hand sprayer (United States Plastic Corp, Lima, Ohio, USA, cat. # 66260). The marked filter paper was air dried and then placed in a 15 × 150-mm Petri dish containing 80 g of moist sterilized soil. The soil was moistened with 6 ml of dH_2O. Undifferentiated termites (workers and soldiers) were placed into the Petri dish and the lid of the dish was replaced and sealed with masking tape. The termites were placed in the incubator described above and allowed to forage freely on the rabbit IgG-marked filter paper for 24 h. The marked filter paper was removed after 24 h and replaced with a clean filter paper moistened with 1.0 ml of dH_2O. The Petri dish containing the termites was again sealed with masking tape and placed back into the incubator described above.

External marking procedure

Termites (workers and soldiers) were marked with rabbit IgG by topically spraying them *en masse* with 1.0 ml of a 5.0 mg/ml reagent grade rabbit IgG solution using a perfume atomizer. The marked termites were then transferred to a Petri dish containing 80 g of sterilized soil as described above.

Internal and external marking procedure

Termites (workers and soldiers) were internally and externally marked by both of the techniques described

above. Again, these termites were maintained as described above.

Unmarked controls

An unmarked group of termites (workers and soldiers) were placed into a Petri dish containing a wetted filter paper and 80 g of sterilized moistened soil and maintained in the incubator described above.

Termite sample preparation

Four termite treatment groups were maintained in the incubator for 35–42 days after marking. We randomly selected 17–20 termites from each treatment group every 2–4 days until all the living termites were removed from each treatment group (note: we only had 6 termites remaining in the Petri dish after 35 days for the internal mark treatment). Individual termite samples were placed into a 1.6 ml micro-centrifuge tube and frozen at –20°C. To prepare frozen termites for ELISA, 1.0 ml tris buffered saline (TBS, pH 7.2) was added to each individual micro-centrifuge tube. Each individual was then macerated with a clean tissue grinder and assayed for the presence of rabbit IgG by the ELISA described below.

Termite marking ELISA

Every termite was analyzed for the presence of the mark by the rabbit IgG specific double antibody sandwich ELISA described by Hagler et al. (1992). Each well of a 96-well ELISA microplate was coated with 100 µl of anti-rabbit IgG (developed in goat) (Sigma Chemical Co., St. Louis, Missouri, USA, cat. # R2004) diluted 1:500 in ddH_2O and incubated overnight at 4°C. The rabbit IgG antibody was discarded and 360 µl of 1% nonfat dry milk in dH_2O was added to each well for 30 min at 27°C to block any remaining non-specific binding sites on the plates. After the nonfat milk was removed, a 100 µl aliquot of a homogenized termite sample was placed in a well of the pretreated assay plate and incubated for 1 h at 27°C. Termite samples were then discarded and each well was briefly rinsed three times with TBS Tween 20 (0.05%) and twice with TBS. Aliquots (50 µl) of anti-rabbit IgG conjugated to horseradish peroxidase (HRP) (Sigma Chemical Co., cat. # A-6154) diluted to 1:1,000 in 1.0% nonfat milk, was added to each well for 1 h at 27°C. Plates were again washed as described above and 50 µl of TMB 1 Component HRP Microwell Substrate (BioFX Laboratories, Owings Mills, Maryland, USA, cat. # TMBW-0100-04) was added to each well. Following a 10 min substrate incubation period, the ELISA optical density reading of each termite sample was measured with a microplate reader set at 650 nm.

Data analysis

Termites serving as negative controls were obtained from our main colony and were also included with each ELISA. Mean (±SD) ELISA absorbance values of the negative controls were calculated for each ELISA plate ($n = 8$ per 96-well ELISA microplate). The ELISA values yielded by the negative controls were virtually identical to the values yielded by the termites collected from the unmarked control treatment. Therefore, termites from each of the four treatment groups were scored positive for the rabbit IgG mark if the absorbance value was three standard deviations above that of the unmarked control treatment mean (Hagler et al., 1992). Bar charts depicting the average (±SD) ELISA absorbance values and proportion of termites positive for rabbit IgG over the course of each experiment were constructed.

Retention of various protein marks on termites

Two separate studies were conducted to determine how rapidly termites acquire an internal mark after feeding on foodstuff (e.g., bait) containing different grades (i.e., reagent grade and technical grade) and concentrations of rabbit IgG.

Reagent-grade rabbit IgG experiment

The termites used in this study were obtained from the main laboratory colony described above. Two equal sized groups ($n \approx 300$) of termites were placed into separate 0.47 l plastic deli cups containing 160 g of sterilized soil moistened with 12 ml of dH_2O. One 0.04 × 1.0 m strip of B-flute corrugated cardboard marked with the highly purified reagent grade rabbit IgG described above was added to each deli cup containing termites. The cardboard strips offered to each termite group differed in the method that they were marked. The first marking treatment consisted of placing a cardboard strip flat onto a sheet of wax paper. Both sides of the cardboard were then sprayed evenly with 5.0 ml (10 ml total) of a 5.0 mg/ml reagent grade rabbit IgG solution using a perfume atomizer. The cardboard strip was air dried for ≈4 h, then rolled up tightly and held in place with a rubber band. The second reagent grade rabbit IgG marking treatment consisted of first, rolling the cardboard strip and holding it in place with a rubber band, and then dunking it in a beaker containing 10 ml of a 5.0 mg/ml reagent grade rabbit IgG solution for 10 s. The soaked cardboard roll was allowed to air dry overnight. Each cardboard roll was then put into a separate deli cup containing ≈300 termites and the cup was placed in the incubator described above.

Twenty termites were randomly collected from each treatment group everyday for 3 days. The first sample was collected 2 h after the termites were first exposed to the marked cardboard. The sampling, sample preparation, and ELISA performed on each termite was identical to the procedures described above.

Technical grade rabbit IgG experiment

This experiment was conducted as above except that technical grade rabbit IgG (Sigma Chemical Co., cat. # I-8140) was used instead of reagent grade rabbit IgG and the cardboard bait was either sprayed or soaked with a 1.0 mg/ml technical grade rabbit IgG solution.

Data analysis

Bar charts depicting the average (±SD) ELISA absorbance values and proportion of termites positive for rabbit IgG over the course of each experiment were constructed. The ELISA data yielded within each marker application treatment (e.g., sprayed and soaked) for each type of mark were analyzed to identify significant differences in ELISA readings over time. These data did not meet the assumption criteria needed for ANOVA (e.g., both non-transformed and transformed ELISA data were not normally distributed and did not contain equal variances) (SigmaStat, Ver. 2.03, Chicago, Illinois, USA). Therefore, a non-parametric Kruskal–Wallis one-way ANOVA on ranks followed by a Dunn's multiple comparison test was used to identify significant differences between the ELISA values yielded over time for each treatment. The ELISA values yielded each day between the sprayed and soaked rabbit IgG treatments were analyzed by the non-parametric Mann–Whitney Rank Sum Test (again, these data did not meet the assumptions needed for the *t* test) to identify significant difference in ELISA values between the two treatments each day.

Results

Long-term retention of protein on termites

Termites were sampled 15 times over 42 days (except for the internally marked termite group which were sampled 14 times over a 35 days period) and assayed for the presence of rabbit IgG by a sandwich anti-rabbit IgG ELISA. The quantitative mean ELISA values yielded by the unmarked termites were consistent throughout the duration of the 42 days of the study, with overall mean ELISA absorbance reading of only 0.040 ± 0.003, thus yielding a positive ELISA control threshold value of 0.049 for individual termites exposed to the various marking treatments. None of the unmarked control termites tested positive for the presence of rabbit IgG (Fig. 1a).

There was a steady decline in the ELISA values yielded by the externally marked termites over the duration of the study. However, every termite scored positive for rabbit IgG for up to 19 days after marking. Overall, 94.7% of the individuals examined yielded a positive ELISA reaction for the presence of the mark (Fig. 1b).

The internally marked termite group yielded 98.1% positive ELISA reactions over the course of the study (Fig. 1c). There was only a slight decay in the ELISA optical density values yielded over time. Only five individual termites tested negative over the duration of the experiment, each of which were sampled 24 days after marking (Fig. 1c).

Every individual termite ($n = 300$) that was doubly marked scored positive for rabbit IgG over the 42-day duration of the experiment. There was only a slight decay in the ELISA optical density values yielded over time (Fig. 1d).

Retention of various protein marks on termites

Reagent grade rabbit IgG experiment

The ELISA reactions yielded by termites exposed to cardboard bait that was either sprayed or soaked with

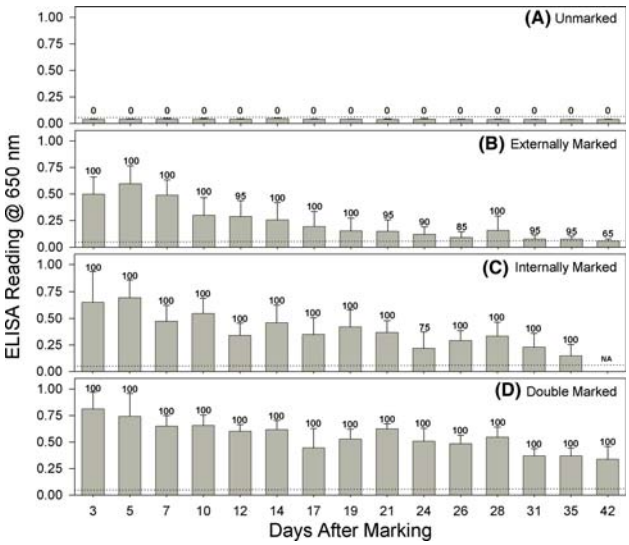

Fig. 1 Retention (mean ± SD ELISA optical density readings) of rabbit IgG on *Heterotermes aureus*. (**a**) Unmarked negative control termites, (**b**) termites externally marked, (**c**) termites internally marked, and (**d**) termites internally and externally marked with rabbit IgG ($n = 17$–20 termites for each mean except for the 35 days internal mark treatment [$n = 6$]). The percentage of termites testing positive for rabbit IgG are given above each *error bar*. The *dotted line* parallel to each *x*-axis is the positive control ELISA threshold value (e.g., mean negative control ELISA value plus 3 SD)

reagent grade rabbit IgG are given in Fig. 2a. The quantitative (color intensity of the ELISA reaction) and qualitative (percentage of individuals marked with rabbit IgG) ELISA reactions were always significantly higher for the termites exposed to the soaked cardboard bait (see Fig. 2a for the test statistic yielded for each paired comparison). The mean ELISA reaction was significantly higher for termites continuously exposed to the soaked cardboard bait for 3 days than those for 2 h or 1 day ($H = 11.51$; $df = 3$; $P = 0.009$). However, the qualitative ELISA results revealed that 95% of the termites became marked with rabbit IgG after only a 2 h exposure period to the soaked cardboard and every termite was positive for rabbit IgG if they were exposed for ≥1 day (Fig. 2a). The mean ELISA reaction was significantly lower for termites exposed to the sprayed cardboard bait at 2 h than those exposed to the bait for 2 or 3 days ($H = 22.12$; $df = 3$; $P < 0.001$) (Fig. 2a). The percentage of these termites scoring positive for the mark ranged from only 5% at 2 h to 85% on day 2 for the sprayed treatment. Overall, over half (56%) of these termites were marked with rabbit IgG; however, it appears that if the termites are exposed to this bait treatment for ≥1 day, then the efficacy of the marking procedure increases to 73.3% (44 out of 60 individuals scored positive for rabbit IgG beyond the 2 h treatment period).

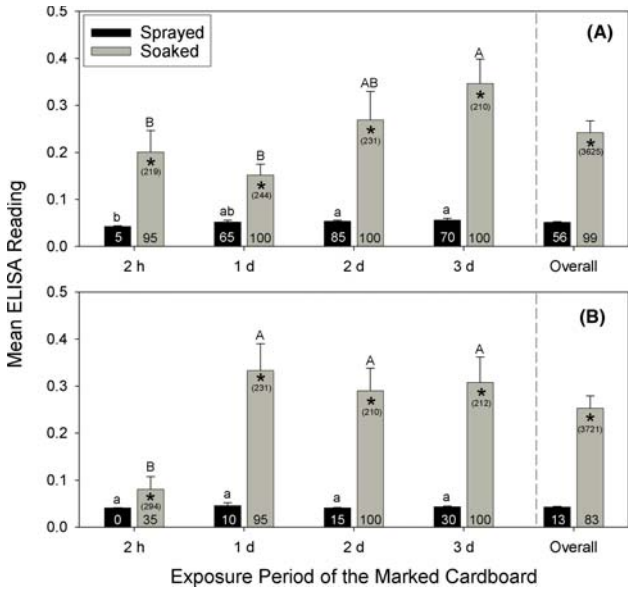

Fig. 2 Mean ± SD ELISA readings for *Heterotermes aureus* exposed to rabbit IgG-marked cardboard bait over a 3 days period. (**a**) Termites exposed to cardboard bait impregnated with reagent grade rabbit IgG and (**b**) termites exposed to cardboard bait impregnated with technical grade rabbit IgG ($n = 20$ for each daily treatment mean, $n = 80$ overall). The percentage of termites testing positive for rabbit IgG is presented within each *shaded vertical bar* for each treatment. Means over time with the same *upper case letter* are not significantly different for termites exposed to the cardboard soaked in rabbit IgG. Means over time with the same *lower case letter* are not significantly different for termites exposed to the cardboard sprayed with rabbit IgG (the Kruskal–Wallis one-way ANOVA test statistics are given in the text). *Asterisks* indicate significant differences between termites exposed to either soaked or sprayed cardboard each day. The number in *parenthesis* below each *asterisk* is the Mann–Whitney *T* statistic for each paired comparison. The *P* value yielded for each paired comparison was $P < 0.001$

Technical grade rabbit IgG experiment

The ELISA reactions yielded by termites exposed to cardboard bait that was either sprayed or soaked with technical grade rabbit IgG is given in Fig. 2b. Again, the quantitative and qualitative ELISA reactions were always significantly higher for the termite group exposed to the soaked cardboard bait treatment (see Fig. 2b for the test statistic yielded for each paired comparison). The mean ELISA reaction was significantly lower for those termites continuously exposed to the bait soaked in the technical grade rabbit IgG for 2 h, with only 35% of these individuals testing positive for the presence of the mark ($H = 30.32$; $df = 3$; $P < 0.001$). However, termites self-marked effectively if they were exposed to the cardboard bait soaked in technical grade rabbit IgG for ≥1 day (59 out of 60 were successfully marked). There were no significant differences in ELISA readings over time yielded by termites exposed to bait sprayed with technical grade rabbit IgG ($H = 6.37$; $df = 3$; $P = 0.095$). Moreover, the qualitative ELISA reactions ranged from 0 to 30% after 2 h and 3 days, respectively (Fig. 2b). Overall, only 13% of the termites tested positive for rabbit IgG.

Discussion

The most effective marker for any given study is often dependent of the type of experiment being conducted (e.g., mark–capture or MRR) or the type of arthropod being studied. To date, internal dyes such as Nile Blue A and Sudan Red 7B have been the most common and effective markers used for mark–capture and MRR-type termite studies (Grace, 1990; Haagsma and Rust, 1993; Su et al., 1993; Forschler and Townsend, 1996; Thorne et al., 1996; Haverty et al., 2000; Baker and Haverty, 2007). For MRR studies, termites were typically fed paper products impregnated with the various colored dyes in the laboratory. In these studies, after about 3 days of continuous feeding on the stained paper, the majority of the termites self-marked. Marked individuals were then released and recaptured at various temporal and spatial intervals for studies of termite dispersal, nest mate behavior, and foraging territories (Su et al., 1988; Jones, 1990; Suárez and Thorne, 2000). For mark–capture studies the dye marker

was usually provided as a marked food source at a centralized location directly in the termite's natural habitat (Evans et al., 1998; Evans, 2002, 2004). In turn, termites self-marked by consuming the marked bait.

The results presented here and in a recent study (Buczkowski et al., 2007) show that protein marking (a.k.a. immunomarking) has potential as a termite marker for both MRR and mark–capture type studies. For MRR-type studies we showed that *H. aureus* retain the mark for weeks, and that protein can be used as an external, internal, or double mark. Also, we showed that termites self-mark rapidly (e.g., $\leq$1 day) after exposure to cardboard bait soaked in either reagent grade or technical grade rabbit IgG. This time frame is much faster than the times reported for termites to acquire a dye mark (Su et al., 1988; Jones, 1990; Suárez and Thorne, 2000). Protein-specific ELISAs are incredibly sensitive and can detect proteins in insect samples at <30 ppb (Jones et al., 2006). Moreover, the anti-rabbit IgG ELISA is a simple and standardized assay and the results can be quantified and saved for analysis. The detection of dyes by visual observation are not quantifiable, tedious if thousands of samples need to be examined, and prone to human error, especially if multiple observers are viewing termites that might only contain a minute trace of colored dye.

We caution that the results presented here are preliminary and more studies are needed to further validate this procedure. Specifically, studies are needed to test the persistence of the mark on various termite species under realistic field conditions. Preliminary field studies indicate that rabbit IgG displays both spatial (e.g., up to 10 m) and temporal (e.g., >8 w) durability on termites at three different field sites located within the harsh Arizona desert ecosystem (Baker, in preparation).

The rationale for testing different purities of rabbit IgG was twofold. First, previous studies showed that a 5.0 mg/ml solution of highly purified reagent grade rabbit IgG was more effective for marking small parasitoids (Hagler et al., 2002). Therefore, we used the best-case-scenario approach for our first study by marking the termites with a relatively high concentration of expensive reagent grade rabbit IgG. The optimal results yielded from this study prompted us to conduct a second study using a lower concentration (e.g., 1.0 mg/ml) of less pure and less expensive technical grade rabbit IgG. Data indicate that cardboard bait soaked in a lower concentration of technical grade rabbit IgG is as effective of a marker for *H. aureus* as cardboard soaked in reagent grade rabbit IgG if the termites are exposed to the bait for a day or more. These data suggest that less expensive technical grade rabbit IgG will be as effective as reagent grade rabbit IgG for large-scale field studies that might require large amounts of protein. Future studies are needed to determine if even lower concentrations of technical grade and reagent grade rabbit IgG can be used to effectively mark termites at a reduced cost.

Studies have shown that the retention of dyes in or on termites varies greatly between both the type of dye applied and the termite species under investigation. The durability of a termite marker can be an asset or a liability, depending on the nature of the experiment. For example, Neutral Red and Nile Blue A have proven useful for long-term studies (e.g., >1 week) for certain termite species, but not for others (Haagsma and Rust, 1993; Oi, 2000; Su et al., 1991, 1993). Conversely, dyes with short retention intervals do not confound studies in which previously marked specimens have been released (Jones, 1990). This study, and the study conducted by Buczkowski et al. (2007), confirmed that both *H. aureus* and *R. flavipes* acquire detectable amounts of rabbit IgG in $\leq$1 day after feeding on paper impregnated with rabbit IgG (Fig. 2). However, *R. flavipes* only retained the internal mark for 4 days, whereas, *H. aureus* retained it for weeks whether it was applied as an internal, external, or double mark (Fig. 1). This difference could be attributed to species to species variability in marking efficiency or to *H. aureus* receiving a greater concentration of the mark. Again, future studies are needed to identify the acquisition and retention of different concentrations of IgG on different termite species.

Another caveat of termite dispersal research is that some studies require two or more distinctive marks (Evans, 2001, 2002). A major advantage of dye markers is that they are available in a variety of colors including red, blue, yellow, green, and black (Evans et al., 1998). Although we only tested rabbit IgG as a termite mark, there are other highly specific proteins and protein-based ELISAs that could be used for termite dispersal studies. For example, rabbit IgG and chicken IgG marks have been used for MRR-type studies to simultaneously examine the intercrop dispersal of the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville (Hagler and Naranjo, 2004) and flight behavior of glassy-winged sharpshooter, *Homalodisca vitripennis* Germer (Blackmer et al., 2004). These distinct proteins could prove useful in combination for termite dispersal studies requiring multiple markers.

Marking termites and other types of social insects efficiently for dispersal studies is problematic because of the potential that any mark can laterally transfer from nest mate to nest mate via trophallaxis or cannibalism. The lateral transfer of a mark from a marked to an unmarked termite could lead to erroneous estimations of dispersal distances and population sizes of termite colonies. Several studies have investigated whether dyes transfer from marked termite donors to unmarked recipients via trophallaxis. Haagsma and Rust (1993) concluded that there was no evidence that Nile Blue or Neutral Red dye transferred from marked to unmarked *R. hesperus*. However, when

large numbers of termites fed on dye-treated paper, the soldiers assimilated the dye, presumably by ingesting food with dye in it that was provided by their colony mates. Grace and Abdallay (1989) also concluded that Sudan Red 7B dye did not readily transfer via trophallaxis from marked to unmarked *R. flavipes*. Buczkowski et al. (2007) showed that *R. flavipes* acquired rabbit IgG within 24 h after exposure to marked bait, but they also showed that trophallactic transfer rates from marked donors to previously unmarked recipients was 51 and 31% to workers and soldiers, respectively after a 72 h exposure period.

Although the protein marking system appears to be effective for marking termites, there are at least two limitations of the procedure. First, the mark cannot be detected visually in the field because it requires an immunoassay to detect for the presence of the non visible mark. Consequently, the detection of mark by ELISA is more complex, difficult, time-consuming and costly than merely detecting a dye on a termite by direct visual inspection. Second, the termite must be crushed prior to the ELISA, so the recaptured insect cannot be returned to the field or used for any other analysis.

In summary, the detection of a rabbit IgG mark by ELISA has potential for MRR and mark–capture termite studies. The mark was easy to apply externally by directly spraying termites with an inexpensive perfume atomizer or internally by feeding termites marked foodstuffs. Finally, the ELISA used to detect the mark is extremely sensitive and specific. In short, rabbit IgG and other types of protein marks have enormous potential for studying various aspects of termite behavior.

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